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POLYMER CHARACTERIZATION BY
ELECTROHYDRODYNAMIC IONIZATION
MASS SPECTROMETRY

FINAL REPORT

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A. Statement of Problem

The program sponsored by this grant (funded jointly with the National Science Foundation Division of Materials Research) was intended to develop and characterize electrohydrodynamic mass spectrometry (EH MS), and to assess and, insofar as possible, to extend its applicability as a tool for the characterization of polymer solutions. Capabilities for determination of polymer molecular weight distributions and interactions with other solutes were of particular interest. Applications to both synthetic and natural polymers were considered.

B. Summary of Most Important Results

Much of the progress achieved can be traced to a study of poly(ethylenimines) (PEIs), and ancillary follow-up studies of model systems. In contrast with earlier results with poly(ethylene glycols), mass spectra of PEI samples appeared to be biased in favor of lower MW oligomers. Total measured ion current per gram of polymer decreased with increasing nominal average molecular weight (\bar{M}). Furthermore, mass spectral estimates of \bar{M} of heavier samples were well short of manufacturer's specifications. This was true for all of a large variety of PEI adduct ions, including ions incorporating protons (up to three, forming $3+$ ions), alkali metals, and transition metals. Significantly, detected transition metal complexes were all singly charged, either as a result of chemical reduction ($\text{Cu}^{2+} \rightarrow \text{Cu}^+$) or ion pairing (Zn^{2+} , Mn^{2+} , and Co^{2+}). Despite optical evidence of interaction with Ni^{2+} , no ions containing this metal were detected. In fact, addition of $\text{Ni}(\text{NO}_3)_2$ suppressed the polymer spectrum, to an extent proportional to the Ni^{2+} concentration.

In initial studies aimed at explaining these phenomena, the extent of sampling bias was assessed by developing an independent, titrimetric means of determining sample average molecular weights (\bar{M}). Additionally, samples were analyzed by vapor phase osmometry and dispatched to other MS labs for comparative MS measurements. These studies revealed comparable bias in several polymer MS experiments, but also indicated that the manufacturer's estimates of \bar{M} were probably somewhat inflated.

Efforts then turned to unravelling the cause of the sampling bias, and attempting to reduce it. Three important, interacting bias mechanisms emerged: ion pairing, solvation, and mass transport. Studies with model compounds (diquaternary ammonium salts, "diquats") revealed that charge reduction via ion pairing in solution could enhance sensitivity, up to the point of neutralization. Because EH MS samples only those ions present in solution, reducing the charge to zero results in desensitization. The extent of ion pairing could be varied by control of the solution composition; extent of ion association increases across the series acetate<nitrite<chloride<nitrate<perchlorate. In earlier studies, we found that enhanced interactions with the extracting field actually improved

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sensitivity when ion charge was shielded by an effective complexing ligand. The diquat and PEI data indicate that ion charge is relatively exposed to solvent interactions in these systems. If the charge is not paired, the resulting high solvation energy increases the potential barrier to field-assisted ion evaporation from the liquid meniscus, thereby reducing sensitivity. If, on the other hand, pairing is so extensive that neutralization occurs, sensitivity will again be reduced. Thus, there should be some optimum, intermediate level of ion pairing that can be achieved through judicious choice of counterion and solvent dielectric (although variation of the latter parameter is difficult).

To further assess the role of solvation in inducing sampling bias against heavier PEIs, EI spectra were obtained for each member of a series of relatively pure, linear, low MW oligomers. Surprisingly, solvation of these oligomers decreased, and sensitivity increased, with increasing molecular weight. By contrast, energy-resolved mass spectra of PEI indicated that some heavy oligomers were sampled with solvent attached, and subsequently evaporated in flight, reducing ion kinetic energy. Evidently, branching of larger oligomers (which were unavailable in pure form for direct testing) must result in enhanced solvent interactions and/or charge exposure. Primary amines constituting multiple end-groups in branched PEIs must play a major role in determining their solvation and complexation chemistry.

An important general conclusion based on this work was the existence of a trade-off between ion-field and ion-solvent interactions in determining ion sampling efficiencies. Both of these interactions increase with ion charge. One (ion-field) enhances sampling, while the other (ion-solvent) impedes it. Attempting to assess their relative importance, we sought a pair of structurally identical model ions differing only in charge. The tris(bipyridyl) complexes of Ru^{3+} and Ru^{2+} seemed to be good candidates, since both are relatively stable in aqueous solution. We found, however, that glycerol (the solvent employed most often in EI MS, due to its low vapor pressure) was capable of rapidly and quantitatively reducing $Ru(bpy)_3^{3+}$ to $Ru(bpy)_3^{2+}$. In fact, by examining a series of transition metal complexes of varying E° (standard reduction potential), we were able to ascertain that the E° of glycerol is near 0.1 V. Direct confirmation of this value by standard electrochemical experiments was not possible, because of the difficulty of monitoring multi-electron organic reductions or oxidations at electrode surfaces. However, the result was consistent with previous observations of the reduction of an aromatic diquat (m-bis(trimethylammonium) benzene) in glycerol. Thus, the importance of the ancillary role of solvent chemistry became clear. This was further evident from detection of acid-catalyzed depolymerization, which occurred when we tried to enhance the abundance of multiply-protonated oligomers by acidifying solutions of PEI. Viewed from an analytical perspective, anything that affects MS signals constitutes a phenomenon that can, in principle, be characterized by MS.

As an alternative means of isolating the effect of charge density on sampling, a homologous series of simple tetraalkyl ammonium salts (methyl through pentyl) was examined. Sensitivity to these salts increased with

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molecular weight through the C₄ homolog (correlating with improved charge shielding and surface activity), then decreased markedly for C₅. Counterion variation established that ion pairing was not responsible for the anomalous decrease. However, changing solvent viscosity (by adding ethylene glycol or lithium salts) did have a marked effect. These observations could be explained on the basis of a mass transport contribution to the sampling mechanism. EH sampling occurs only from the tip of the EH emitter. To be sampled, analyte must reach that tip from the sample reservoir. At least three mechanisms of mass transport contribute: convection (flow), diffusion, and migration. Of these, only the first has no dependence on a sample-dependent diffusion coefficient. If two materials are removed with equal ease, the one which diffuses more slowly will be depleted from the sampling region, resulting in a time-dependent sampling bias against that component. We have modeled this behavior for a simple mixture, accurately predicting observed time dependences. With further refinement, the model may provide a means of assessing diffusion coefficients of individual oligomers sampled from a complex mixture.

Both the mass-transport and solvation contributions to PEI sampling bias can be mitigated by using less viscous solvents. Bias was reduced when sampling from mixed water-glycerol solutions; water-thiodiethanol mixtures gave even better results.

Even in cases of severe bias, the use of an internal standard can correct for sampling bias, providing good quantitative results. While standards for PEI were not available, good estimates of relative stability constants were possible in a related study of alkali metal complexes of 18-crown-6. This study exploited the exceptional sensitivity of MS; weak adducts undetected by more conventional methods could be investigated.

Less extensive studies were undertaken with a number of other polymer materials. Anionic polyelectrolytes (poly(styrene sulfonate) (PSS) and styrene/acrylic acid co-polymers) proved difficult to sample, although a monomer impurity and a proton-bound dimer of PSS were noted with interest. By contrast, small oligomers of a cationic polyelectrolyte with shielded charge (a phenyl iodonium oligomer) was sampled with excellent sensitivity, as was Oxypruf E (a polyethoxylated pyrazole). In the latter case, reasonably accurate MW distributions were obtained, as was information about contaminants and competitive cation interactions. Quantitative results with the related materials Oxypruf P (a polypropoxylated pyrazole) and Oxypruf 6 (a polypropoxylated hydrazine) were less accurate, probably because these polymers were only slightly soluble in the solvent employed (glycerol). Even for these materials, however, useful information about impurities and competitive ion interactions was obtained.

Preliminary studies with "natural polymers" (oligopeptides) have centered on development of "dynamic" EH MS; monitoring reactions proceeding within the MS ion source. Introducing the enzyme carboxypeptidase Y as a reagent in a solution containing an oligopeptide analyte causes slow hydrolysis of the peptide, from the carboxy terminus. By monitoring the mass spectrum during hydrolysis, both the peptide sequence and the kinetics of the reaction can be discerned. Significant effort has been (and is

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being) devoted to discovery of a solvent system providing good MS sensitivity while not excessively interfering with normal enzymatic kinetics. Even if rates measured differ from those observed in simple aqueous systems, intermediates observed should reasonably reflect mechanistic details. Some effort has been directed at development of an ion source that can accommodate pure aqueous solutions (with no low-volatility, viscous co-solvents). This would also facilitate use in a liquid chromatography/MS system.

Finally, companion studies have assessed and compared the invasiveness of EI MS and the more common fast atom bombardment (FAB) MS technique. A series of organic dyes susceptible to beam-induced reduction in FAB were examined by EI and FAB in a variety of matrices and under varying conditions of FAB primary beam energy and flux. In no case was there evidence of reduction in the EI spectra. By contrast, for several samples, the beam-damaged reduction product was the principal analyte ion in the FAB spectrum obtained under standard operating conditions. However, by lowering flux, increasing energy, and/or using a less reductive solvent (thioglycerol or m-nitrobenzyl alcohol instead of glycerol), the beam damage could be mitigated substantially.

C. List of Publications

1. "Electrohydrodynamic Mass Spectrometric Studies of Some Polyether-Cation Complexes," V.F. Man, J.D. Lin, and K.D. Cook, J. Am. Chem. Soc. 107, 4635 (1985).
2. "Polymer Mass Spectrometry: The Problem and its 'Solution'," K.D. Cook, J.H. Callahan, V.F. Man, and K. Hool, in Specialty Instrumentation (28th Oak Ridge Nat. Lab. Conf. on Anal. Chem. in Energy Tech.); Laing, Ed.; Lewis: Chelsea, MI, 1986; p. 101.
3. "Electrohydrodynamic Mass Spectrometry," K.D. Cook, Mass Spec. Rev. 5, 467 (1986).
4. "Polymer Mass Spectrometry," K.D. Cook, in Encyclopedia of Polymer Science & Engineering, 2nd ed.; Mark, Bikales, Overberger, and Menger, Eds.; Wiley: New York, 1987; Vol. 9, p. 319.
5. "Mass Transport Effects in Electrohydrodynamic Mass Spectrometry," J.H. Callahan, K. Hool, J.D. Reynolds, and K.D. Cook, Int. J. Mass Spectrom. Ion Proc. 75, 291 (1987).
6. "Factors Affecting the Sampling of Poly(ethylenimines) by Electrohydrodynamic Mass Spectrometry," K.D. Cook, J.H. Callahan, and V.F. Man, Anal. Chem. 60, 706 (1988).
7. "Glycerol-Induced Reduction in Electrohydrodynamic Mass Spectrometry," J.H. Callahan, K. Hool, J.D. Reynolds, and K.D. Cook, Anal. Chem. 60, 714 (1988).
8. Physical Properties of Matrices used for Fast Atom Bombardment," K.D. Cook, P.J. Todd, and D.H. Friar, Biomed. Environ. Mass Spectrom. 18, 492 (1989).

The following publications are in press.

9. "Isotope Label Scrambling in Alkyl Cations: A Mechanistic Proposal," A.A. Tuinman and K.D. Cook, Rapid Comm. Mass Spectrom., in press.
10. "An Electrohydrodynamic MS Study of Factors Affecting the Efficiency of Matrix-Assisted Desorption Ionization," K.D. Cook, in Ionization from Organic Solids; Sundqvist, Ed.; Wiley: New York, in press.

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D. Participating Personnel

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